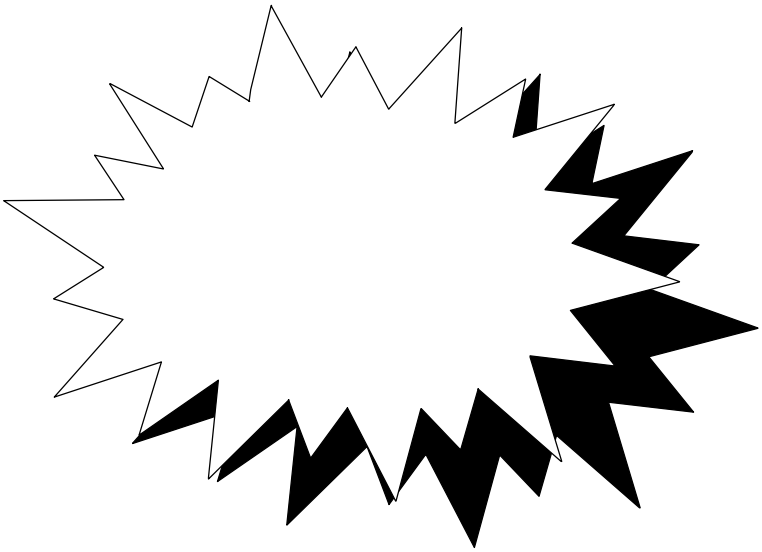

Chemical Analysis and Testing Task

Laboratory Analytical Procedure

LAP-007

Procedure Title: ***Preparation of Dilute-Acid Pretreated Biomass***

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Preparation of Dilute-Acid Pretreated Biomass

Laboratory Analytical Procedure #007

1. Introduction

1.1 Lignocellulosic biomass feedstocks, typically, contain large quantities (55-75% by weight) of carbohydrates that are polymers of 5- and 6-carbon sugars. Most or all of these carbohydrates can be converted to ethanol via biotechnology. For bioconversion to occur, however, the polymers must first be broken down into low-molecular-weight, or, essentially, monomeric, sugars, and it is generally accepted that enzymatic hydrolysis is the preferred pathway. Nevertheless, the native (indigenous) cellulose fraction of the carbohydrates is recalcitrant to enzymatic breakdown; therefore, a pretreatment step is required to render it amenable to enzyme attack. Although various pretreatment techniques are under investigation, a batch, dilute sulfuric acid prehydrolysis method is currently employed at NREL to prepare pretreated biomass for fermentation research.

2. Scope

2.1 This procedure describes the batch dilute-acid pretreatment method employed at NREL to prepare pretreated biomass for fermentation research in the Ethanol Project. Biomass is pretreated in a pressure reactor at 160°C for approximately 10 minutes using dilute sulfuric acid. The procedure has been developed as a result of several years of pretreatment research at NREL and has been found to result in prehydrolyzate (the liquid phase resulted from a dilute-acid pretreatment run) of satisfactorily high xylose yield and high pretreated solids enzyme digestibility. Depending on research goals, the pretreated biomass may or may not need to be washed. The operating conditions used in the procedure, however, have not been optimized to maximize the xylose yield, enzyme digestibility, or the ethanol yield that can be obtained through fermentations. Furthermore, the optimal pretreatment conditions are expected to be substrate specific.

2.2 All analyses shall be performed according to the guidelines established in the Ethanol Project Quality Assurance Plan (QAP).

3. References

3.1 NREL Ethanol Project CAT Task Laboratory Analytical Procedure #001, "Standard Method for Determination of Total Solids in Biomass".

3.2 Grohmann, K., Torget, R., and Himmel, M. 1985. "Optimization of Dilute Acid Pretreatment of Biomass." Biotech. Bioeng. Symp. No. 15. 59-80.

3.3 Grohmann, K., Himmel, M., Rivard, C., Tucker, M., Baker, J., Torget, R., and Graboski, M. "Chemical-Mechanical Methods for the Enhanced Utilization of Straw." 1984. Biotech. Bioeng. Symp. No. 14. 137-157.

4. Significance and Use

4.1 Pretreatment is necessary before the carbohydrates in lignocellulosic biomass can be converted to ethanol via biotechnology. Dilute-acid pretreatment is one technique that has been extensively investigated and found to be effective.

5. Apparatus

5.1 Parr Instrument Company pressure reactor system, including either a 2-gal or a 1-L reactor vessel made of Carpenter 20 Cb-3 material, with a matching mixer, jacket electrical heater, and temperature/mixing control unit that comprises a thermocouple, all supported on a movable metal stand. Attached to the head plate of the reactor vessel are a mixer shaft, a pressure gauge, a vent, a valve for chemical injection, a pressure rupture disc, and a thermowell.

5.2 Hoist (required only for the 2-gal system).

5.3 High pressure pump (HPLC pump, Beckman model 110, or equivalent).

5.4 Balance(s).

5.5 pH meter. (**Note: A pH meter must be used if pretreatment of a new type of biomass substrate is performed. If pretreatment of the substrate has been performed previously, pH paper, as specified in Paragraph 6.7, can be used in place of the pH meter.**)

5.6 Stopwatch.

5.7 Tachometer.

6. Reagents and Materials

6.1 Sulfuric Acid, 72% (w/w) (12.00 ± 0.02 M or specific gravity 1.6389 at 15.6°C/15.6°C).

6.2 pH 1.00 and 2.00 calibrating buffers.

6.3 Feedstock substrate: Feedstock substrate is milled to an appropriate size (normally -3 mm [-1/8 in.]). To prevent possible charring in the reactors, the substrate is often further sieved to remove -60 mesh particles. Upon preparation, the moisture content is determined, following Laboratory Analytical Procedure #001, Standard Method for Determination of Total Solids in Biomass.

6.4 Two 3- to 5-gal pails (required only if the 2-gal system is used) or two 1-L beakers (required only if the 1-L system is used).

6.5 A sink suitable for an ice bath to be prepared for cooling the 2-gal reactor vessel after a pretreatment run (required only if the 2-gal system is used) or an 8-L plastic bucket (required only if the 1-L system is used).

6.6 Two 50-mL graduated cylinders (required only if the 2-gal system is used) or a 10-mL and a 50-mL graduated cylinder (required only if the 1-L system is used).

6.7 pH paper covering pH range 1 to 2. (**Note: If the operator prefers using pH meter to pH paper, this paragraph can be skipped.**)

6.8 Prepare the following four items only if separation of prehydrolyzate and pretreated solids or washing of pretreated solids is required.

6.8.1 A Buchner funnel of 6-L capacity (required only if the 2-gal system is used) or 600-mL capacity (required only if the 1-L system is used).

6.8.2 A 2-L vacuum flask and an aspirator (required only if the 1-L system is used).

6.8.3 A filter cloth (a white cotton bed sheet can be cut into size for use) of approximately 3 ft x 3 ft (required only if the 2-gal system is used) or a Whatman No. 5 filter paper (required only if the 1-L system is used).

6.8.4 pH paper covering pH range 2 to 6. (**Note: If the operator prefers using pH meter to pH paper, this paragraph can be skipped.**)

7. ES&H Considerations and Hazards

7.1 Before any work proceeds, review the appropriate SOP on pretreatment system. It is required that new personnel be trained by an experienced personnel prior to conducting any pretreatment experiment.

7.2 Follow all applicable NREL Laboratory Specific Hygiene Plan guidelines.

7.3 72% H_2SO_4 is very corrosive and must be handled carefully.

7.4 Apparatus in this procedure are heated to elevated temperatures, use caution to avoid burns.

7.5 High pressure is generated in the reactor. Take precautions to avoid unexpected venting of the reactor contents.

8. Procedure for Using 2-gallon Parr Reactor System

8.1 Feedstock Substrate and Chemicals Preparation and Other Preparative Work

8.1.1 Weigh an appropriate amount (approximately 480 g dry weight) of feedstock substrate in a 3- to 5-gal pail. Refer to Paragraph 10.1.1 for an example of the feedstock substrate weight.

8.1.2 Targeting a 10% solids level in the reactor, use a 50-mL graduated cylinder to weigh an appropriate amount of 72% sulfuric acid. The weight of the acid is determined according to calculations shown in Paragraph 10.1.2. (**Note: The target acid level is to obtain a prehydrolyzate pH of 1.3-1.5. Since the prehydrolyzate pH is not known prior to the run, the acid concentration to use has to be preestimated. NREL experience has shown that, for a hardwood substrate, 0.73 wt% acid in the liquid phase in the reactor is generally acceptable. For a herbaceous substrate, 0.88 wt% acid is generally acceptable. Following pretreatment, the prehydrolyzate pH should be tested to determine if the acid concentration used is acceptable. If not so, it must be adjusted and the run repeated until the target pH is met.**)

8.1.3 Targeting at the solids level in the reactor specified above, weigh an appropriate amount of deionized water in the second 3- to 5-gal pail. The weight is determined according to calculations shown in Paragraph 10.1.3.

8.1.4 Thoroughly flush the HPLC pump intake and delivery lines with deionized water (not the water prepared in Step 8.1.3) and discard the effluent. Following flushing, turn off the pump.

8.1.5 Prepare 15 mL deionized water in the second 50-mL graduated cylinder.

8.1.6 Place the pump intake tubing in the acid from Step 8.1.2 and run the pump for approximately 5 minutes at 6 mL/min, directing the effluent from the delivery line back into the acid graduated cylinder. Turn off the pump at the end of the 5-minute period.

8.1.7 Quantitatively transfer the weighed feedstock substrate and water, respectively, from Steps 8.1.1 and 8.1.3 into the reactor (using some of the water to rinse the feedstock substrate pail).

8.1.8 Manually mix the reactor contents.

8.1.9 Close the vent and the chemical injection valves on the reactor head plate, followed by closing the head plate.

8.1.10 After the reactor is securely closed, open the vent valve.

8.2 Pretreatment Run

8.2.1 Use the hoist to load the reactor into the heater, making sure the vent line and the rapture disc relief line on the head plate are facing away from the operator.

8.2.2 Insert thermocouple in thermowell and attach the drive unit.

8.2.3 Turn on the mixer motor, setting the rpm to be about 220 ± 20 (use tachometer to verify rpm). Allow reactor contents to mix at ambient temperature for 5 minutes. Proceed to complete the next step during the 5-minute mixing time.

8.2.4 While Step 8.2.3 is in progress, attach the HPLC pump delivery line to the reactor chemical injection valve.

8.2.5 Following the 5-minute mixing time in Step 8.2.3, turn on the heater and set the temperature controller set-point to 50°C.

8.2.10 As soon as the reactor temperature reaches 159.5°C, turn on the HPLC pump at a flow rate of 6 mL/min, start the stopwatch, followed by opening the chemical injection valve. Monitor time and reactor temperature throughout this period. Prepare a control chart of the reactor pressure at 160°C. The temperature needs to be within 160 " 1°C or the experiment must be repeated.

8.2.11 When the acid graduated cylinder is almost empty (after approximately 4 minutes for a hardwood substrate or 5 minutes for a herbaceous substrate), note the elapsed time and add the deionized water from Step 8.1.5 slowly (to prevent air bubbles from entering the pump intake line) to the acid graduated cylinder to flush out acid in the line to the reactor.

8.2.12 As soon as the deionized water in the acid graduated cylinder has all entered the intake tubing, turn off the pump, close the chemical injection valve, and disconnect the pump delivery line from the valve.

8.2.13 At about the 11.5-minute mark for a hardwood substrate (or 12-minute mark for a herbaceous substrate), turn off the mixer motor, disconnect the drive unit, and move the drive unit and the thermocouple out of way.

8.2.14 Use the hoist to lift the reactor out of the heater and, when the elapsed time reaches 10 minutes plus half of the acid injection time noted in Step 8.2.11, lower the reactor into the ice bath to quench the reaction.

8.2.15 Rotate the mixer shaft manually to assist in cooling uniformly the reactor contents.

8.2.16 When the pressure in the reactor drops to below 5 psig, check with the thermocouple that was removed from the thermowell in Step 8.2.13 to ensure the temperature in the reactor is 95°C or lower. Then, open the vent slowly to relieve the remaining pressure in the reactor.

8.2.17 Upon fully relieving the pressure in the reactor, use the hoist to lift the reactor out of the ice bath and set it upright on the flat surface next to the sink.

8.2.18 Remove the reactor head plate, making sure little or no solids are attached to the mixing unit.

8.2.19 (This step can be skipped if the feedstock substrate has previously been used and the appropriate amount of acid to use is known.) Determine the pH of the prehydrolyzate with the pH meter (the meter should be calibrated with the pH 1.00 and 2.00 calibrating buffers). If the pH is not between 1.3-1.5, repeat the run using an adjusted amount of 72% sulfuric acid. Otherwise, proceed to the next step.

8.2.20 If it is required to separate prehydrolyzate and pretreated solids or to wash the pretreated solids, proceed to Step 8.3. Otherwise, transfer the reactor contents to an appropriate container and label the container properly for storage in a refrigerated area.

8.3 Post-Pretreatment Sample Handling

8.3.1 Set the 6-L Buchner funnel on a clean, dry 3- to 5-gal pail on the floor.

8.3.2 Place the filter cloth over the funnel.

8.3.3 Filter the contents of the reactor through the funnel. Catch the prehydrolyzate in the pail and squeeze the residual prehydrolyzate off the pretreated solids.

8.3.4 Store the prehydrolyzate, sealed and properly labeled, in a refrigerated area.

8.3.5 If washing of the pretreated solids is required, proceed to the next step. Otherwise, store the pretreated solids, sealed and properly labeled, in an appropriate container in a refrigerated area.

8.3.6 Add deionized water to the Buchner funnel to wash the pretreated solids and monitor the pH of the filtrate using pH paper until it reaches the desired pH. Mix the solids with washing water occasionally to ensure effective washing.

8.3.7 Squeeze the residual water off the pretreated solids.

8.3.8 Store the washed pretreated solids, sealed and properly labeled, in an appropriate container in a refrigerated or frozen area.

9. Procedure for Using 1-L Parr Reactor System

9.1 Feedstock Substrate and Chemicals Preparation and Other Preparative Work

9.1.1 Weigh an appropriate amount (approximately 60 g dry weight) of feedstock substrate in a 1-L beaker. Refer to Paragraph 10.2.1 for an example of calculating the feedstock substrate weight.

9.1.2 Targeting a 10% solids level in the reactor, use the 10-mL graduated cylinder to weigh an appropriate amount of 72% sulfuric acid. The weight of the acid is determined according to calculations shown in Paragraph 10.2.2. (**Note: The target acid level is to obtain a prehydrolyzate pH of 1.3-1.5. Since the prehydrolyzate pH is not known prior to the run, the acid concentration to use has to be preestimated. NREL experience has shown that, for a hardwood substrate, 0.73 wt% acid in the liquid phase in the reactor is generally acceptable. For a herbaceous substrate, 0.88 wt% acid is generally acceptable. Following pretreatment, the prehydrolyzate pH should be tested to determine if the acid concentration used is acceptable. If not so, it must be adjusted and the run repeated until the target pH is met.**)

9.1.3 Targeting at the solids level in the reactor specified above, weigh an appropriate amount of deionized water in the second 1-L beaker. The weight is determined according to calculations shown in Paragraph 10.2.3.

9.1.4 Thoroughly flush the HPLC pump intake and delivery lines with deionized water (not the water prepared in Step 9.1.3) and discard the effluent. Following flushing, turn off the pump.

9.1.5 Prepare 15 mL deionized water in the 50-mL graduated cylinder.

9.1.6 Place the pump intake tubing in the acid from Step 9.1.2 and run the pump for approximately 5 minutes at 6 mL/min, directing the effluent from the delivery line back into the acid graduated cylinder. Turn off the pump at the end of the 5-minute period.

9.1.7 Quantitatively transfer the weighed feedstock substrate and water, respectively, from Steps 9.1.1 and 9.1.3 into the reactor (using some of the water to rinse the feedstock substrate beaker).

9.1.8 Manually mix the reactor contents.

9.1.9 Close the vent and the chemical injection valves on the reactor head plate, followed by closing the head plate.

9.1.10 After the reactor is securely closed, open the vent valve.

9.2 Pretreatment Run

9.2.1 Place the reactor into the heater, making sure the vent line and the rupture disc relief line on the head plate are facing away from the operator.

9.2.2 Insert thermocouple in thermowell, attach the drive unit, connect mixer shaft seal cooling water lines, and allow cooling water to flow through the lines (with discharge line directed to the drain).

9.2.3 Turn on the mixer motor, setting the rpm to be about 175 ± 20 (use tachometer to verify rpm). Allow reactor contents to mix at ambient temperature for 5 minutes. Proceed to complete the next step during the 5-minute mixing time.

9.2.4 While Step 9.2.3 is in progress, attach the HPLC pump delivery line to the reactor chemical injection valve.

9.2.5 Following the 5-minute mixing time in Step 9.2.3, turn on the heater and set the temperature controller set-point to 50°C.

9.2.6 The heater will be turned off automatically when the reactor temperature reaches 50°C, but the temperature will continue to rise to about 75°C. Allow the reactor to be heated at about 75°C and mixed for 10 minutes to remove trapped air.

9.2.7 At the end of the 10-minute period, close the reactor vent and adjust the controller set-point to 150°C.

9.2.8 While the reactor is being heated and before the temperature reaches 158°C, prepare an ice bath in the 8-L plastic bucket. (It takes about 20 minutes for the reactor temperature to reach 158°C.)

9.2.9 When the reactor temperature reaches 158°C, change the controller set-point to 160°C.

9.2.10 As soon as the reactor temperature reaches 159.5°C, turn on the HPLC pump at a flow rate of 6 mL/min, start the stopwatch, followed by opening the chemical injection valve. Monitor time and reactor temperature throughout this period. Prepare a control chart of the reactor pressure at 160°C. The temperature needs to be within 160 " 1°C or the experiment must be repeated.

9.2.11 When the acid graduated cylinder is almost empty, note the elapsed time and add the deionized water from Step 9.1.5 slowly (to prevent air bubbles from entering the pump intake line) to the acid graduated cylinder to flush out acid in the line to the reactor.

9.2.12 As soon as the deionized water in the acid graduated cylinder has all entered the intake tubing, turn off the pump, close the chemical injection valve, and disconnect the pump delivery line from the valve.

9.2.13 At about the 9.5-minute mark, turn off the mixer motor, disconnect the drive unit, detach the cooling water lines of the mixer shaft seal, and move the drive unit and the thermocouple out of way.

9.2.14 Lift the reactor out of the heater and, when the elapsed time reaches 10 minutes plus half of the acid injection time noted in Step 9.2.11, lower the reactor into the ice bath to quench the reaction.

9.2.15 Rotate the mixer shaft manually to assist in cooling uniformly the reactor contents.

9.2.16 When the pressure in the reactor drops to below 5 psig, check with the thermocouple that was removed from the thermowell in Step 9.2.13 to ensure the temperature in the reactor is 95°C or lower. Then, open the vent slowly to relieve the remaining pressure in the reactor.

9.2.17 Upon fully relieving the pressure in the reactor, lift the reactor out of the ice bath and set it upright on the bench.

9.2.18 Remove the reactor head plate, making sure little or no solids are attached to the mixing unit.

9.2.19 (This step can be skipped if the feedstock substrate has previously been used and the appropriate amount of acid to use is known.) Determine the pH of the prehydrolyzate with the pH meter (the meter should be calibrated with the pH 1.00 and 2.00 calibrating buffers). If the pH is not between 1.3-1.5, repeat the run using an adjusted amount of 72% sulfuric acid. Otherwise, proceed to the next step.

9.2.20 If it is required to separate prehydrolyzate and pretreated solids, or, to wash the pretreated solids, proceed to Step 9.3. Otherwise, transfer the reactor contents to an appropriate container and label the container properly for storage in a refrigerated area.

9.3 Post-Pretreatment Sample Handling

9.3.1 Set the 600-mL Buchner funnel on the 2-L vacuum flask and connect the aspiration line.

9.3.2 Place a sheet of Whatman No. 5 filter paper in the funnel.

9.3.3 Apply vacuum to filter the contents of the reactor through the funnel. Catch the prehydrolyzate in the vacuum flask. Wait until dripping of prehydrolyzate stops.

9.3.4 Store the prehydrolyzate, sealed (in a bottle, for example) and properly labeled, in a refrigerated area.

9.3.5 If washing of the pretreated solids is required, proceed to the next step. Otherwise, store the pretreated solids, sealed and properly labeled, in an appropriate container in a refrigerated area.

9.3.6 Apply deionized water to the Buchner funnel to wash the pretreated solids and monitor the pH of the filtrate using pH paper until it reaches the desired pH. Mix the solids with washing water occasionally to ensure effective washing.

9.3.7 Wait until dripping of prehydrolyzate stops.

9.3.8 Store the washed pretreated solids, sealed and properly labeled, in an appropriate container in a refrigerated or frozen area.

10. Calculations

10.1 Example calculations for the 2-gallon Parr reactor system:

10.1.1 Weight of feedstock substrate: assuming the moisture content of the feedstock substrate to be 22.0%, then a convenient substrate weight of 615 g can be used. (The dry weight of the substrate is 615 x (1-22.0%) = 480 g.)

10.1.2 Weight of 72% sulfuric acid: since it is known that the target slurry solids level is 10% and the target acid concentration in the reactor liquid phase is 0.73 wt% (assuming the feedstock to be a hardwood), using the numbers from Paragraph 10.1.1, the weight of 72% sulfuric acid is obtained as follows:

$$\frac{615\ x\,(1-22\%)}{10\%}\ x\,(1-10\%)\ x\,0.73\%\ x\ \frac{1}{72\%} = 43.8\ g$$

10.1.3 Weight of dilution water: to obtain a 10% slurry in the reactor using the numbers from Paragraph 10.1.1, the total weight of the liquid phase in the reactor needs to be:

$$\frac{615\ x\,(1-22\%)}{10\%}\ x\,(1-10\%) = 4320\ g$$

$$4320 - 615\ x\ 22\% - 43.8 - 15 = 4120\ g$$

Since the liquid phase (assuming the dilute-acid extractives present in the feedstock substrate to be negligible) in the reactor is comprised of (1) the moisture in the feedstock substrate, (2) the dilution water, (3) the 72% sulfuric acid added, and (4) the acid line flushing water, the weight of the dilution water required is:

10.2 Example calculations for the 2-L Parr reactor system:

10.2.1 Weight of feedstock substrate: assuming the moisture content of the feedstock substrate to be 22.0%, then a convenient substrate weight of 80.0 g can be used. (The dry weight of the substrate is: 80.0 x (1 - 22.0%) = 62.4 g.)

10.2.2 Weight of 72% sulfuric acid: since it is known that the target slurry solids level is 10% and the target acid concentration in the reactor liquid phase is 0.73 wt% (assuming the feedstock to be a hardwood), using the numbers from Paragraph 10.2.1, the weight of 72% sulfuric acid is obtained as follows:

$$\frac{80.0\ x\ (1-22\%)}{10\%}\ x\ (1-10\%)\ x\ 0.73\%\ x\ \frac{1}{72\%} = 5.69\ g$$

$$\frac{80.0\ x\ (1-22\%)}{10\%}\ x\ (1-10\%) = 562\ g$$

10.2.3 Weight of dilution water: to obtain a 10% slurry in the reactor using the numbers from Paragraph 10.2.1, the total weight of the liquid phase in the reactor needs to be:

Since the liquid phase (assuming the dilute-acid extractives present in the feedstock substrate to be negligible) in the reactor is comprised of (1) the moisture in the feedstock substrate, (2) the dilution water, (3) the 72% sulfuric acid added, and (4) the acid line flushing water, the weight of the dilution water required is:

$$562 - 80.0\ x\ 22\% - 5.7 - 15 = 523\ g$$

11. Precision and Bias

11.1 The precision of Parr reactor operation relies largely on operators strictly adhering to the procedure described above. It also relies on the stable performance of instruments, including balance(s), pH meter, pressure gauge, stopwatch, tachometer, and thermocouple (with display) used in the operation. In addition, the stable performance of the HPLC pump used for acid injection affects the precision of the operation. Thus, in addition to the operator's quality skills that must be ensured, the instruments and equipment involved must be maintained in reliable working conditions.

Statistical analysis of a limited database of six replicate runs (using the same substrate and operating conditions by six different operators) shows that the coefficient of variation for the contents of the major components in the pretreated solids (glucan and Klason lignin) and in the prehydrolyzate (glucose, xylose, and acid-soluble lignin) were all below 10%. The coefficient of variation for enzyme digestibility of the pretreated solids was also below 10%.

11.2 Systematic errors may be incurred in preparing dilute-acid pretreated biomass if any of the instruments used is not properly calibrated. It is thus of critical importance to ensure proper calibration of the instruments. Periodic calibration of the various instruments should be performed in accordance with the time intervals recommended by the instrument manufacturers.

12. Quality Control

12.1 *Reported significant figures:* Report prehydrolyzate pH to two decimal places, if using a pH meter and one decimal place if using pH paper. Monitoring of temperature to 0.1°C during Parr reactor runs is required. All weight determinations should include three significant digits.

12.2 *Replicates:* During normal operations, only properly trained operators will be involved and only single runs are required. For QA/QC verification or for training purposes, multiple runs using the same feedstock substrate and the same operating conditions are required either by the same operator or by different operators. The compositions of prehydrolyzate and pretreated solids and/or the digestibility or fermentability of the pretreated solids will then be compared and statistically analyzed to ensure consistency.

12.3 *Blank:* Not applicable

12.4 *Relative percent difference criteria:* Not applicable for single runs. When duplicate runs are conducted, the relative percent difference for glucose, xylose, and Klason lignin of prehydrolyzate and pretreated solids (where applicable) should be no greater than 5%. For acid-soluble lignin, ash, digestibility, and fermentability, the relative percent difference should be no greater than 10%.

12.5 *Method verification standard:* Not applicable

12.6 *Calibration verification standard:* Not applicable

12.7 *Sample size:* Typically, 1-L and 2-gal Parr reactor runs, respectively, use 60 and 480 g dry weight of the prepared feedstock substrate.

12.8 *Sample storage:*

12.8.1 Feedstock: Depending on total solids level and length of storage, the feedstock substrate is recommended to be stored in ambient conditions (when total solids content is above 88% and, thus, no risk of deterioration is involved) or refrigerated (when total solids content is below 88% for up to 1 month storage) or frozen (when total solids content is below 88% for prolonged storage).

12.8.2 Pretreated products: Prehydrolyzate or pretreated solids samples should be refrigerated. It is further recommended to store pretreated solids in acidified conditions. If washing and prolonged storage of a washed pretreated solids sample is required, freezing is recommended.

12.8.3 *Standard storage:* Not applicable

12.8.4 *Standard preparation:* Not applicable

12.8.5 *Definition of a batch*: Not applicable

12.8.6 *Control charts:* Prepare a control chart of reactor pressure at 160EC for each pretreatment run.